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The HIV envelope protein, gp120 binds to the CD4 cell surface protein and thereby the virus enters T4 helper lymphocytes. This process of virus entry might be associated with the presentation of unique epitopes of the gp120 that become revealed only as a result of virus binding to its target cell. The purpose of this research has been to identify cryptic epitopes that become accessible due to induced conformational changes that result from that complex formation of gp120 with CD4. Thus CD4/gp120 complexes have been used to immunize mice and monoclonal antibodies have been generated. At least 7 antibodies have been identified as having preferred affinity for the above complex. These antibodies are now being characterized. They represent 3 distinct epitopes as has been determined by competitive ELISA assays. Moreover, using a syncytium assay they have been evaluated for their neutralizing activity.

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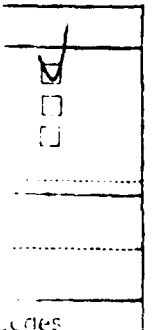

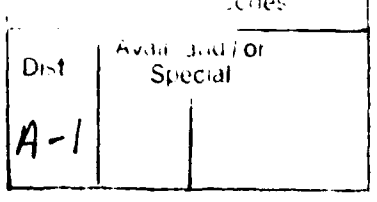

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INTRODUCTION:

The development of an effective vaccine for the prevention of AIDS is a goal that needs no special justification. A number of approaches have been attempted over the last decade since the AIDS epidemic has been recognized and the virus HIV identified as the cause of this devastating disease. Major efforts have focused around the viral envelope glycoprotein gp120 which is noncovalently attached to the particle via the membrane-associated gp41.

The structure of gp120 has been extensively analyzed and the glycomoieties, the disulfide arrangements [1] and variable *versus* conserved domains have been defined [2]. The most prominent region of this protein has been the Primary Neutralizing Domain (PND) that consists of the V3 loop (residues 266-301, [3]). Antibodies to this epitope have been found to be neutralizing in a highly strain-selective manner [4]. Nonetheless, there are regions within this loop that can elicit broad spectrum neutralization [5, 6]. A second target within the gp120 has been the areas involved in CD4 recognition [7]. Obviously, antibodies that bind these areas are anticipated to interfere with the virus association to its target cells and thus prevent infection. This epitope is believed to be complex being constructed from discontinuous segments of the gp120. In any case, a desired objective would be to produce a subunit vaccine that would provide broad spectrum protection against HIV infection. Unfortunately, neither the V3 loop nor the CD4 binding domains of gp120 appear to satisfy this goal.

Therefore, the search for novel more effective epitopes for the production of subunit vaccines is still extremely important and relevant. The strategy proposed in this study has been to identify new aspects of the gp120 molecule that could serve this purpose. The approach adopted here is based on the assumption that gp120 can assume a variety of conformations. In doing so gp120 would thus present different aspects of itself during various phases of the disease. For example, gp120 when associated with gp41 may be quite distinct from soluble gp120 or the CD4 bound protein. The notion that the envelope undergoes conformational rearrangements is supported by the fact that the N terminal domain of gp41 becomes available for membrane fusion only after the gp120 attaches to CD4 [8].

Recently, this line of thought has been further elaborated upon in studies that focus on the idea that after the binding of gp120 to CD4 there must exist a series of events that are related to viral *entry* rather than binding *per se*. Celada et al [9] and Healey et al [10] both focus on this point and develop mAbs against CD4 that have neutralizing activity yet are by definition not of the classical type of Leu3A or OKT4A namely, the new antibodies do not interfere with the complex formation.

The major effort this first year of the present study has been to produce novel mAbs that are unique in that they have preferred affinity for the gp120/CD4 complex.

BODY:

Materials:

Recombinant gp120 produced in a baculovirus expression system was purchased from American Bio-Technologies. Recombinant soluble CD4 (produced in CHO cells) as well as a anti-V3 loop mAbs (NEA-9305) were obtained from DuPont. The rest of the reagents used were standard and of analytical grade.

Methods:

V8 protease digests: Recombinant gp120 (10µg/ml) was incubated 24-48 hours in 50mM NH_4HCO_3 pH7.8/0.05% SDS/1µg *Staphylococcus aureus* V8 protease at 37°C. Addition of ß-mercaptoethanol (5%) to the reaction generated a unique set of fragments.

Protein blot analyses: Intact or proteolysed protein fragments were separated on SDS-polyacrylamide gels as previously described and transferred electrophoretically in an apparatus that generates a gradient electric field to compensate for differences in molecular mass of the peptides being blotted [11]. Either nitrocellulose or charged modified nylon membranes were used as the blotting matrix. Blots were quenched either with 1% milk solutions in 50mM Tris buffered saline pH7.5 (TBS) or 5% hemoglobin solutions in the same. Probes such as biotinylated lectins or mAbs were routinely diluted in a quenching solution and their presence revealed by using enzyme conjugated second reagents and standard detection reactions (for a general review on the above blotting procedures see [12]).

ELISA assays: Costar EIA/RIA 96 well plates (N3590) were coated with 50µl of gp120/CD4 complex (5µg gp120:2.5µg CD4/ml) or gp120 (5µg/ml) or CD4 (5µg/ml) in TBS overnight at 4°C. The plates were then washed in TBS and blocked with 3% BSA in TBS for 1 hour at room temperature (RT). The wells were rinsed and the mAbs at various dilutions in 0.3%BSA/TBS were added to them and incubated at RT for 2-3 hours. The wells were then washed with TBS and the second antibody (alkaline phosphatase conjugated goat anti-mouse antibody [Sigma, A-0162]) was added (1:1000 in 0.3%BSA/TBS) and incubated for 1 hour at RT. After washing the wells they were reacted with p-nitrophenyl phosphate (1mg/ml in 1M diethanolamine buffer pH9.8 / 0.5mM $MgCl_2$) and read at 405nm.

Production of monoclonal antibodies: The production of mAbs was performed using standard procedures immunizing

the mice (Balb/C) first in complete Freund's adjuvant and boosted with in-complete adjuvant. The mice were allowed to rest 3 weeks before their i.v. boost and splenectomy followed three days later. NS-1 myeloma cells were used for PEG induced fusion and HAT medium for selection. Clones were obtained by limited dilutions [13].

mAb purification: mAbs were isolated from ascites fluids taken from pristane primed mice. The standard caprylic acid followed by ammonium sulfate precipitation procedure was used [13].

Biotinylation of mAbs: mAbs (~1mg/ml) were dialysed overnight against 0.1M $NaHCO_3$ (pH was not adjusted). Biotinamidocaproate N-hydroxysuccinimide ester (BNS, [Sigma B-2643]) was added (3µg/100µg protein) and incubated for 4 hours at RT, followed by overnight dialysis against TBS.

Syncytium assay: The formation of syncytia between vaccinia infected BSC1 cells and CEM cells was performed essentially as was described by Ashorn et al. [14] In principle, BSC1 cultures were infected with recombinant vaccinia (5pfu/ml) expressing cell surface gp120 (VPE16 provided by Dr. Bernie Moss, NIH). These were then mixed with CEM cells in the presence of variable amounts of mAbs and incubated for different times. The degree of syncytia formation was monitored and thus the extent of neutralization potential for the various mAbs was estimated.

Results:

Characterization of V8 proteolyzed gp120: Digestion of gp120 with *S. aureus* V8 protease produces 5 major fragments detectable on western blots probed with ConA (see schematic summary in Figure 1).

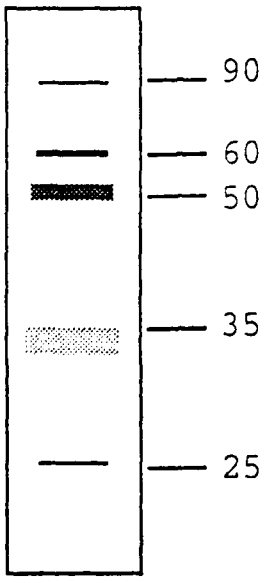

Figure 1: Schematic Diagram of V8 Proteolyzed gp120.

Western blots of V8 proteolyzed gp120 were probed with anti V3 mAbs, ConA or sCD4. To the left is a summary of the bands which can be detected. Depending on the degree of digestion and the probed used the presence and the intensity of each band can vary.

The conditions used (see Methods) were found to generate reasonably reproducible patterns although in view of the fact that the simple sum of the molecular weights of the fragments yields a total mass of over 250kDa the pattern received can only be a set of overlapping fragments rather than a true terminal digest. The reasons for this are unclear. More aggressive denaturation might however, lead to over digestion and the generation of numerous unresolved small fragments. Western blots of transferred V8 digested gp120 were analyzed with: ConA, commercial monoclonal anti gp120 antibodies, and with sCD4 itself. The signal for sCD4 overlay of proteolyzed gp120 is weak but reproducible. In view of the fact that it is believed that the critical CD4 binding domain is in the COOH aspect of gp120, combined with the response to the V3 loop antibody, we can postulate a gross map of the gp120 with respect to some of the fragments we resolve (Figure 2).

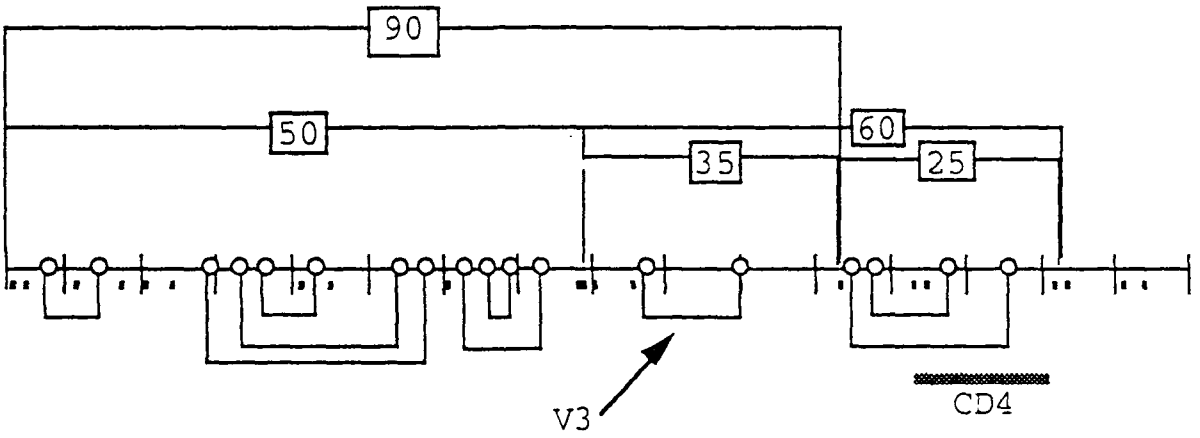

Figure 2: Map of gp120 V8 Fragments.

In the above diagram the circles indicate cys residues and the disulfide arrangements are given. The black dots indicate the approximate positions of the glutamic acid residues (V8 cleavage sites).

In figures 1 and 2 the 90kDa fragment contains the V3 loop but does not bind CD4. This band is lost after reduction with ß-mecaptoethanol and thus is probably the result of an early cleavage. The 60 kDa reacts with both anti V3 loop mAb and sCD4. The 50kDa does not bind CD4 nor anti V3 loop mAbs and is lost after reduction, indicating that it might be a series of small fragments held together via disulfide bridges. The 35 kDa fragment is the smallest containing the V3 loop and the reaction with anti V3 loop mAbs seems to intensify after reduction. The position of the 25kDa fragment can only be speculated.

Production of mAbs against V8 fragments of gp120: Three mice were injected with V8 cut gp120 and given a series of boosts. Initially, it appeared that only a weak response developed and thus additional boosts were given.

As can be seen in Table 1 this increased the response of the mice against gp120.

TABLE 1: Analysis of Anti V8/gp120 Sera.

dilution of sera	I		II	
	gp120	V8/gp120	gp120	V8/gp120
1/50	674	-	1270	817
1/100	598	-	1220	624
1/200	310	-	1000	471
1/400	182	-	695	319
1/800	139	-	391	168
1/1600	-	-	229	93
1/3200	-	-	116	41
1/6400	-	-	77	31

The above table describes the response of one mouse that was immunized with gp120 V8 fragments. As can be seen, the titer is much improved in the second bleed.

The above mouse was selected and sacrificed on June 11, 1992. Its spleen contained $3x10^{-8}$ cells and these were fused in three aliquots with NS-1 myeloma cells and 2/3 of the cells were plated and processed as two separate fusions into a total of 13 96-well plates (the third aliquot was frozen down for future studies).

The hybridoma cultures were screened against gp120 and proteolysed gp120. Only five clones were found to be positive and one of these was lost in the process of cloning. The remaining four were found to be IgM of very low affinity and low titer in the ascites fluids obtained. It appears that the digested gp120 is a poor antigen and mice should be more aggressively immunized before new fusions are attempted. Unfortunately, in the mean time the remaining two mice that were immunized with the proteolyzed gp120 have died.

Production of CD4/gp120 complexes: CD4 and gp120 were used to generate their corresponding complex in a number of procedures. The sCD4/gp120 complex could be demonstrated via ELISA assays. In principle, either gp120 or CD4 were plated and then the corresponding counter part was used to probe the plate. Subsequently, the plates were probed with either anti-gp120 or OKT4 followed by an alkaline phosphatase conjugated anti-mouse. Moreover, OKT4A was used to ascertain that the complex that was formed was, as expected, unable to bind this monoclonal (see Table 2). Finally, once conditions were established preparations of complex were made for immunizations.

TABLE 2: Analyses of gp120/CD4 Complexes.

gp120 µg/ml	OKT4A	OKT4	CD4 µg/ml	OKT4A	OKT4
10.0	27	640	5.0	206	729
5.0	23	773	2.5	135	492
2.5	27	313	1.25	42	291
1.25	29	116	0.60	22	138

The above table is an example of the analysis of the formation of gp120/CD4 complex on the ELISA plate used in this study. gp120 was applied to the wells using concentrations as indicated. Then CD4 (5µg/ml) was incubated with the gp120 and the wells were subsequently probed with the antibodies as indicated (numbers represent ODx10^{-3}). The right half of the table is a control in which CD4 was plated and the same antibodies were tested.

Production of anti-complex mAbs: Three mice were originally immunized against the complexed CD4/gp120. After the initial immunization two of the mice died and it was concluded that the MES (N-methylmorphiline) which is used as a preservative in the sCD4 might be toxic for the mice. A second set of three mice were then immunized with complex that had been extensively dialyzed. These mice and a remaining original mouse developed good responses against both CD4 and gp120 (see Table 3).

TABLE 3: Analysis of Anti Serum to CD4/gp120 Complex.

dilution of sera	gp120 5mg/ml	CD4 5mg/ml	CD4/gp120 5mg/ml
1/50	964	803	973
1/100	941	746	942
1/200	861	696	859
1/400	690	629	755
1/800	571	516	610
1/1600	462	463	502
1/3200	290	336	284
1/6400	299	297	250

The above depicts the response of a mouse that was immunized with the CD4/gp120 complex.

The mouse described in Table 3 was that with the highest titer and therefore was given an i.v. boost and was sacrificed for splenectomy and the fusion for hybridoma production. The spleen was found to extremely large (>4 x 10^8 cells were obtained). Therefore, 4 aliquots of ~10^8 cells were taken separately. Each was fused with NS-1 cells. Two fusions were processed in parallel (the remaining cells were frozen down for future studies). A total of 1170 clones were obtained and after 10 days of culture, the media were screened against CD4/gp120 complex in an ELISA assay. 147 clones were found positive and

these were then re-screened against CD4/gp120 complex as well as CD4 and gp120 separately. Of the original clones only 81 continued to secrete antibodies and of these 15 were selected for future characterization. Of these 13 have proven to be stable and have been injected into mice for the successful production of ascites fluids.

Ascites fluids of all 13 mAbs described were produced and lyophylized and sent to Captain Tom VanCott. These were received and analysed for their ability to bind CD4, gp120 and their complex. The mAbs were tested in ELISA assays, FACS analyses and BIAcore measurements. A collection of ten mAbs were identified as being interesting for further analyses (see Table 4).

Table 4:mAbs Produced Against gp120/CD4 Complex.

	mAb	subclass	gp120/CD4	gp120	CD4
1	JG-1.3	IgG1	+++	-	+/-
2	JG-4.5	IgG1	+++	+++	-
3	JG-7.19	IgG1	+++	-	+/-
4	JG-8.1	IgG1	+++	-	+/-
5	JG-9.5	IgG1	+++	-	++
6	JG-10.1	IgG1	+++	-	-
7	JG-25.3	IgG1	+++	-	++
8	JG-30.5	IgG1	+++	-	++
9	JG-40.1	IgG1	+++	+++	-
10	JG-76.5	IgG1	+++	-	+++

Results obtained in Tel Aviv corresponded well with those at Walter Reed except for three mAbs (JG-1.3, JG-7.19 & JG-8.1) which appeared at Walter Reed to have affinity for CD4 as well as the complex, thus these mAbs will have to be further characterized. The antibodies were biotinylated and competitive ELISA assays were performed to determine epitope overlaps. In this manner six epitopes were defined (see Table 5).

Table 5: Epitope Classification by Competitive ELISA.

	1	4	7	8	9	10	25	30	40	76
1	+	-	+	+	-	-	-	-	-	-
4	-	+	-	-	-	-	-	-	-	-
7	+	-	+	+	-	-	-	-	-	-
8	+	-	+	+	-	-	-	-	-	-
9	-	-	-	-	+	-	+	+	-	-
10	-	-	-	-	-	+	-	-	-	-
25	-	-	-	-	+	-	+	+	-	-
30	-	-	-	-	+	-	+	+	-	-
40	-	-	-	-	-	-	-	-	+	-
76	-	-	-	-	-	-	-	-	-	+

Neutralization potential of the mAbs: The various mAbs were tested for their neutralizing activity using the syncytium assay described in the Methods. In this procedure BSC1 cells are infected with VPE16 recombinant vaccinia virus so that they now express cell surface gp120. These cells are mixed with CEM cells and can therefore form syncytia. Syncytia formation is inhibited by the neutralizing antibodies. At least seven antibodies have been found to be neutralizing to various degrees. Of particular interest is the fact that the mAb JG-10.1 which is complex specific by all the assays performed thus far in both labs, at Tel Aviv and at Walter Reed, is neutralizing. As these results are not rigorously quantified as yet they must be further developed before the efficiency of the system can be fully appreciated.

CONCLUSIONS:

The major target of this project focuses on the postulate that gp120 changes its conformation upon its association with CD4. The molecular ramification of such induced conformational change would be the appearance of structures that are otherwise hidden, i.e. cryptic epitopes. One would hope that antibodies directed towards these unique structures might interfere with the biology of the gp120.

The experiments performed here illustrate that such epitopes are a reality. The closest results published thus far are those of Celada [9] where he and his colleagues isolated one mAb that could correspond to any one of the three CD4 epitopes that we have identified (this is assuming that clones JG-1.3, JG-7.19 & JG-8.1 indeed bind CD4). Moreover, clone JG-10.1 is genuinely unique with no parallel reported thus far. This clone is the first and most direct example of a complex dependent mAb as it has excellent affinity for the complex and no detectable binding to CD4 or gp120. Fortunately, it also is able to inhibit in the syncytium assay described in this report.

A major task that has been more fully appreciated in the course of this work has been the problem of quantitatively characterizing the mAbs that we have produced and qualitatively mapping their epitopes. The collaboration with Capt. VanCott has already proven to be extremely useful for the Tel Aviv laboratory and is sought to be intensified. Towards this goal we are scaling up the production of purified mAbs to supply the U.S. lab so to coordinate an effective research plan. Moreover, new experiments are being designed so to increase our understanding of the biology and the effects of these unique reagents.

During the course of the next year the mAbs will be used to evaluate the range of their specificity, their ability to neutralize field isolates and serological studies of bloods

from Israeli cohorts as well as other HIV infected individuals will be conducted. Such studies have already been initiated in collaboration with the Israeli hemophiliac center at the Chaim Sheba Medical Center at Tel Hashomer.

Additional fusions will be performed using the mice that have already been immunized with the CD4/gp120 complex as well as novel complexes and protocols that will be tested.

Finally we believe that the application of the Pharmacia BIAcore technology is extremely exciting and very important to the progress and insurance of high quality work for this study in particular. We therefore request to purchase a BIAcore for the Israeli laboratory. This will in no way hinder the extent of the collaboration with the Walter Reed but quite the contrary. It is our belief that having a BIAcore system functioning in both labs will enable a more fruitful dialogue between the two groups. In view of this we are submitting a request to this point as an Appendix to this report.

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APPENDIX:

Request for Purchase of a

Pharmacia BIAcore System

Justification:

During the course of the first year of this project we have had to analyze thousands of hybridomas in order to focus on a collection of unique monoclonal antibodies. Once identified, these mAbs are now being characterized; determining their affinities, mapping their epitopes and comparing their epitope similarities or differences with other mAbs, establishing the extent of antigenic cross reactivity and of course evaluating their potential as neutralizing agents of HIV infection and potential use as immunotherapeutics. These tasks require an extensive amount of work and should be performed quantitatively in a most rigorous fashion in order to produce meaningful and useful information of the highest quality.

As more mAbs are to be produced in this project the logistics become compounded in their complexity as new mAbs obviously must be compared with those already characterized.

When this project proposal was initially submitted the BIAcore was barely in its infancy. Last year when the project was approved I had the opportunity of visiting the BIAcore facility headed by Captain VanCott at the Walter Reed / Rockville, MD and was very impressed with what appeared to be the ideal system for the efficient analysis of the mAbs I hoped to produce. During the course of this last year I have discussed the progress of BIAcore technology and applications with Capt. VanCott and at the Keystone meeting last March was delighted and excited to learn that this system is in fact much more effective that I had ever imagined.

In order to evaluate the utility of this system in the analysis and characterization of the specific mAbs that we have already successfully isolated, the Tel Aviv laboratory has initiated a genuine collaboration with Capt. VanCott and sent him samples of ascites fluids of 13 mAbs and several samples of purified mAbs, all these to be tested in the BIAcore system at Rockville. In August this year I spent a few days with Capt. VanCott in Rockville having therefore the opportunity to appreciate the power of the BIAcore "on hand".

It is clear that not only are many of the tasks I am in the process of conducting at Tel Aviv amenable to BIAcore analyses and far more quantitative using this system, there

are numerous parameters that would be impossible or highly impractical to obtain otherwise, with out this system. In view of these developments and the success we have already had in generating what we believe to be extremely unique and useful mAbs, we are convinced that purchase of a BIAcore for the Tel Aviv laboratory would be exceedingly useful and therefore essential and would enable a quantum step ahead for generating the high quality reagents we desire to produce.

In view of the importance we foresee in this acquisition of the BIAcore I have initiated discussions with both Pharmacia in Israel and their headquarters in Germany as well as with a number of local funds to obtain some support for this purchase. In summary, I believe that I could obtain at least $50,000 towards the purchase of a BIAcore from local sources. Moreover there is a chance that I could come to some agreement with Pharmacia to obtain special conditions for this purchase as this would be the first BIAcore in this area of the world.

As is indicated, the price that we have been quoted for the complete system is $156,000.- (see quote attached). We would anticipate that running costs for chips, pumps etc. would be $10,000.-/year although this estimate might need adjustment.

Therefore, we request $116,000.- for the purchase of a BIAcore system a soon as possible (Pharmacia Germany has promised ***immediate*** delivery). We would ask for an additional $10,000 for next year's (year 3 of the contract) budget for the running costs of the BIAcore.

We sincerely hope that the funds can be found to assist us in the purchase of this essential piece of equipment.

GAMIDOR Ltd.
Bio Medical Services & Supplies

גאמידור בע"מ
הספקה תשרותים ביו-רפואיים

Ordering nr.	article	price in US $
BR-1000-01	BIAcore™ System	

biosensor-based, automated system for real-time Biospecific Interaction Analysis (BIA) without using any label. Qualitative as quantitative data (e.g. association rate and dissociation rate constants, affinity constants etc.) are available within short times(seconds to minutes). BIAcore™ was developed for research purposes and pilot-steps of routine assays (e.g. development of ELISAs, receptor/ligand assays, process control, method development for affinity chromatography etc.) In combination with the SensorChip CM5, BIAcore™ shows an extremly high reproducibility of the results (CVdose normally < 5%).

Quantity delivered :

- BIAcore™ processing unit
- one and one sample racks type A and B (type A for 57 sample vials with 7, 9 u. 16 mm ø o.d. and type B for 60 sample vials with 9 mm ø o.d.
- Integrated µ-fluidic cartidge (IFC)
- BIAlogue™ systemsoftware for controlling BIAcore™

Gamidor Ltd. P.O. Box 120 Savyon, 56915
Add: 54 Harei Yehuda St. Givat Savyon
Tel: 03-5351505 , 5351205 Fax. 03-346573

גאמידור בע"מ, ת.ד. 120 סביון 56915
כתובת: רחוב הרי יהודה 54, גבעת סביון
טל: 03-5351505 , 5351205 פקס: 03-346573

- controlling instrument Compaq 386/20e with 4 MB RAM, 40 MB hard-disk, VGA coulored monitor, 80387 Co-processor
- keyboard (engl.), MS DOS version 3.31(engl.), MS -
- Windows version 3.0, MS Excel version 1.0
- Laser printer, type HP Laserjet II

Initial issue of consumables:

4 x Sensor Chip CM5 (3 pcs/package each)
Amine Coupling Kit for 50 immobilisations
Surfactant P 20
Autosampler vial kit
all cables, wires and tubings necessary for the installation

US $ 156.000.--

Specifications :

Processing Unit:

Programmable parameters:
timed events : transfer, mix, inject, report points
Flow rate : 1 - 100 µl/min
Flow cell selection (1 to 4) and automatic sample loop (5 and 45 µl selection on the IFC; integrated microvalves are controlled by pressurized air

required sample volumes :	30 µl for 1-4 µl- injections
	75 µl for 5-50 µl-injections
Dead volume :	≤ 0.5 µl
Reproducibility :	>95 %, C.V. ≤ 5%
max sample positions	2 x 96
Mains supply	100-120 V or 220-240 V, ± 10 %
Power consumption	580 VA (max)
Weight :	45 kg
Dimensions :	76 x 35 x 60 cm (l x w x h)

Sensor Chip CM5:

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The sensor chip is a three layer composition of glass, gold and carboxymethylated dextran, optimized for concentrating and immobilising of biomolecules. The four possible, parallel channels (2.1 x 0.55 x 0.05 mm) will be formed by docking the Sensor Chip to the integrated μ-fluidic cartridge. Each channel can be used several times (life-time depends on the stability of the immobilised ligand), up to 50 to 100 times. The sensor chip is fixed on to a polystyrene support frame and protected from dust and mechanical damage by a plastic sheath. Each sensor chip is packaged in a hermetically sealed packet under nitrogen atmosphere.

Total: US $ 156.000.--

This price information includes packing charges and shipment, installation and introduction to BIAcore™ in theory and practice

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GAMIDOR Ltd.
Bio Medical Services & Supplies

גאמידור בע"מ
הספקה תשרוקים ביו-רפואיים

גבעת סביון, 23 באוג' 1992

לכבוד:
ד"ר ג'וני גרשוני
אימונולוגיה וחקר הסרטן
אונ' תל-אביב
רמת אביב

הצעת מחיר מס. מפ-773

ה ס פ ק: Pharmacia Biosystems

המערכת: ראה דפים מצורפים.

תנאים כלליים:
1. המחיר הוא מן המלאי בדולר ארה"ב.
2. המחיר אינו כולל מע"מ.
3. זמן אספקה: 6 שבועות.
4. תוקף ההצעה: 60 יום.

ב ב ר כ ה,

לולי גורביץ
המחלקה המדעית

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